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**Effect of thermo-sonication condition on carotenoid yield and its antioxidant activity**

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**Abstract**

The carotenoids can be extracted from many plants by using many techniques in order to obtain the highest yield. However, the extraction condition also be affected to the quality of the extracts. This work aimed to study the effect of thermo sonication on carotenoid yield as well as its antioxidation activity. The Fuzzy analytical method was used to be applied to gain the best condition which gave both the quantity and quality. The carotenoids were extracted from industrial tomato waste using a thermo-sonication method at different conditions namely extraction temperatures of 30, 50, or 70 °C and extraction times of 10, 30, or 50 min. The High Performance Liquid Chromatograph (HPLC) chromatogram showed that the crude extract was composed of *trans*-lycopene as well as  $\beta$ -carotene. The extraction temperature of 50 °C gave the highest carotenoid content, while extraction times of 30 and 50 min were comparable. The antioxidant activity of crude extract was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and Inhibition of lipid peroxidation. Nevertheless, the antioxidant activities of the extract were not in agreement with a carotenoid yield which extracted at 30 and 70 °C gave a higher level than that at 50 °C. The performance index was calculated by triangular fuzzy analytical method using 4 criteria, which were *trans*-lycopene and  $\beta$ -carotene content, as well as the 2 measurements of antioxidant activity, with the weight of 30:20:25:25, respectively. The extraction condition that yielded the highest performance index was the extraction temperature of 70 °C for 10 min.

**Keywords:** Lycopene, Thermo-sonication, Fuzzy analytical method, DPPH radical scavenging activity, Inhibition of lipid peroxidation

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**1. Introduction**

Carotenoids are natural pigment giving the yellow, orange or red color in fruit, root crops and vegetable. The carotenoids have a backbone structure of isoprene compound which some are linked cyclic end groups, namely  $\beta$ -carotene, while some are similar to one or no cyclic end groups, namely lycopene [1]. The  $\beta$ -carotene is a common carotenoid which has a cyclic portion at both head and tail ending [1]. Lycopene is a predominant carotenoid compound with an acyclic isoprenoid structure having no oxygen and containing 11 conjugated and 2 non-conjugated double bonds [2-5]. It has more potential antioxidant activity in singlet oxygen quencher activity than all carotenoids ( $\beta$ -carotene,  $\alpha$ -carotene) and also  $\alpha$  tocopherol [6-7]. The most carotenoids are heat stable, however, they can be oxidized or isomerized easily. Lycopene is found in naturally as all-*trans*-isomers; however, it can also be degraded or isomerized into *cis*-isomers [8]. Such structural damage has an effect on its health benefits and antioxidant activity. Thus, lycopene extraction must be controlled to reduce degradation or isomerization during the extraction process.

The lycopene can be extracted by many techniques in order to obtain the highest lycopene yield and short time consumption. Poojary and Passmonti [3] reported that the optimum condition for extraction of lycopene from tomato pulp waste (seed removal) was the extraction temperature of 20 °C for 40 min, with the use of 25 % acetone in n-hexane as an extract solvent and at the ratio of 40 mL solvent per solid. Barros et al. [9] used supercritical extraction at 400 bar and 50 °C to extract lycopene from avocado pulp and tomato pomace with

higher 80% recovery of oil and the lycopene yields. Hatami et al [10] reported that the maximum lycopene from tomato processing by-products gained by Supercritical carbon dioxide at temperature of 80 °C at 50 MPa with the ration of peel: seed was 70:30. However, many researches have been chosen the extraction condition which achieve the highest level of carotenoids yield. Whereas, some researches have played attention to the extract yield and its antioxidant activity. Kahili et al [11] extracted lycopene and  $\beta$ -carotene using supercritical CO<sub>2</sub>, found that even though the high temperature, pressure and flow rate of CO<sub>2</sub> gave high yield, but mild extraction conditions gave a higher antioxidant capacity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

The ultrasound-assisted extraction method has been reported that it is useful for extracting many bioactive compounds. Eh and Teoh [2] reported that ultrasound-assisted method enhances the lycopene yield, improve extraction rates, and also reduce required extraction temperatures. Moreover, Xu and Pan [3] revealed that the utilization of an ultrasound to assist in extracting lycopene from red grapefruit led to a higher yield and reduced the extraction time when compared to conventional methods. Ladole et al. [12] used ultrasound together with enzymatic extraction to release lycopene from tomato peel and found that the extraction at 3% (w/w) enzyme co-immobilized amino-functionalized magnetic nanoparticles (AMNPs), pH 5.0, together with an ultrasound power of 10W for 20 min gain a maximum lycopene.

Tomatoes are one of the plants/fruits containing high levels of carotenoid. They are processed into many products, such as soups, juices, purees, pastes, and ketchup [13]. It has been reported that the thermo processing can increase lycopene content in the tomato product [14]. Processing of tomatoes into various products produces a large amount of waste. The composition of tomato waste depends on the type of processing. In the production of tomato paste, industrial waste consists of tomato seeds, peel, and pulp [13]. The tomato waste has been usually used as an animal feed or fertilizer. Moreover, the tomato pomace has been reported to be used as an ingredient for spicy tomato crisp and a tomato crisp [15]. Furthermore, this industrial tomato waste provides a useful material for bioactive compound. Sengkhampan and Phonkerd [16] extracted the phenolic compound from the tomato waste and found that its activity of crude extracted was affected by flavonoid content. Moreover, it also been used as a material for pectin extracted in which the lycopene was trapped in the obtained pectin [17].

As describe above, many researches were pointed to higher carotenoid or lycopene yield with different methods more than paying attention to its antioxidant activity. But in this research study attempted to find the best extraction condition with giving high quality (antioxidant activity) by using (triangular) Fuzzy Analytical Method (FAM). FAM is a mathematical technique which is used to eliminate vague decisions by integrating the results (assessment scores) of all criteria for each condition into the overall performance index [18]. By using a triangular fuzzy grade and its membership function, this method gives a numerical data which is not vague and imprecise. This index number can also be used for comparison and determination the suitable conditions. FAM is approached as an evaluation tool for assessment system, such as for food product quality control [19] and for supply chain assessment [20,21]. In the recent year, FAM has been developed for sensory evaluation by calculating the obtained sensory scores into numerical value of food products namely drinks from bread [22], Dahi (Indian yoghurt) powder [23], and rice-cracker [24]. Moreover, the FAM has been used for decision of optimum pectin extraction condition [25].

This present study investigated thermo sonication condition with gave higher carotenoid yields from tomato waste derived from the tomato paste industry and also determined its antioxidant activity using DPPH radical scavenging assay and inhibition of lipid peroxidation. The suitable condition which achieved the highest level of carotenoids yield together with high antioxidant were evaluated by using Fuzzy Analytical Method.

## **2. Materials and methods**

### *2.1 Materials*

Tomato waste was obtained from the tomato paste industry located in NongKhai Province, Thailand. The tomato waste was dried at 60 °C for 24 h. Then, it was ground, vacuum-packed and stored at -18 °C before further experimentation.

### *2.2 Thermo-sonication condition*

The tomato waste powder (5 g) was added to 95% ethyl alcohol at a solid/solvent ratio of 1/20 w/v. The bottles were placed in a sonication bath (Elmasonic S70H, ElmaHans Schmidbauer GmbH & Co. KG, Singen, Germany) with ultrasound frequency of 37 kHz and sonicated at 30, 50, and 70 °C for 10, 30, and 50 mins each. The extraction temperature was be controlled in the range of 2 °C at the appointed temperature and the solvent was not be exchanged below 2 mL.

After that, the mixtures were cooled and centrifuged (SORVALL BioFuge STRATOS, Thermo Fisher Scientific, Osterode, Germany) at 6000 rpm for 20 mins. The supernatants were collected and hexane containing 0.1% BHT was added 2 times in order to separate the hydrophobic portion. The separation was performed twice. Sodium sulphate anhydrous was added in order to remove some of the water in the extracts. The hydrophobic portion was then exposed to a rotary evaporator to remove the hexane. The crude extract was adjusted to 5 mL by *n*-hexane for control the concentration of the extract and stored at -80 °C for further analysis.

### 2.3 Determination of lycopene content

The lycopene content in the crude extract was determined using a UV-visible spectrophotometer (T80 UV/vis spectrophotometer, PG Instrument Ltd., Leicestershire, England) at 503 nm. The lycopene content was calculated using an extinction coefficient in hexane ( $17.2 \times 10^4 \text{ mol cm}^{-1}$ ) and expressed as mg/100 g of tomato waste powder [3].

### 2.4 Antioxidant activity assay

#### 2.4.1 DPPH radical scavenging activity

DPPH radical scavenging activity of the crude extracts was measured according to the protocol described by Erken et al. [26] with some modifications. In brief, the crude extracts were diluted with ethyl acetate. A 1.5 mL portion of the mixture was added to 1.5 mL of 0.2 mM DPPH in a methanolic solution and then left to stand for 30 mins in the dark. Absorbance of the mixture was measured using a UV-visible spectrophotometer (T80 UV/vis spectrophotometer, PG Instrument Ltd., Leicestershire, England) at 515 nm and recorded as  $A_{\text{sample}}$ . In a control mixture, ethyl acetate was used instead of crude extract and absorbance of the control mixture was recorded as  $A_{\text{control}}$ . DPPH radical scavenging activity was calculated with the following Equation 1:

$$\text{Radical scavenging activity (\%)} = (A_{\text{control}} - A_{\text{sample}}) \times 100 / A_{\text{control}} \quad (1)$$

#### 2.4.2 Ferric thiocyanate test

A ferric thiocyanate test was conducted according to the protocol described by Erken et al. [26] with some modifications. A 100  $\mu\text{L}$  portion of the crude extract was added to 65  $\mu\text{L}$  of linoleic acid in 2.5 mL of absolute ethanol and 2.5 mL of 0.04 M phosphate buffer. A control sample was prepared by using distilled water instead of crude extract. The mixture was then adjusted to 10 mL using distilled water and left at 40 °C for 24 h. The 100  $\mu\text{L}$  of mixture was then taken and diluted with 4.7 mL of 75% ethanol. After that, 100  $\mu\text{L}$  of 30% ammonium thiocyanate and 100  $\mu\text{L}$  of  $\text{FeSO}_4$  were added to the test solution. The mixture was left to stand for 30 min, and the absorbance of the mixture was then determined using a UV-visible spectrophotometer at 500 nm (T80 UV/vis spectrophotometer, PG Instrument Ltd., Leicestershire, England). Antioxidant activity was expressed as inhibition lipid peroxidation (%), calculated using the following Equation 2:

$$\text{Inhibition lipid peroxidation (\%)} = 100 \times (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \quad (2)$$

where  $A_{\text{control}}$  and  $A_{\text{sample}}$  were an absorbance of the mixture control and sample, respectively.

### 2.5 High performance liquid chromatography analysis

The carotenoid content in the crude extract was determined by using a Shimadzu High Performance Liquid Chromatograph (HPLC) (Shimadzu LC-20A, Kyoto, Japan) according to the protocol described by Kha et al. [6] with some modifications. The crude extracts in amber vials were diluted with chloroform containing 0.1% Butylated hydroxytoluene (BHT) and then injected into polymeric C18 reverse phase columns (25 cm x 4.6 mm, 5  $\mu\text{m}$  id.) (Ascentis® C18, Supelco Analytical, Bellefonte, USA). The mobile phase was acetonitrile/dichloromethane/methanol (50:40:10 v/v/v) with a flow rate of 1 mL/min. A photodiode array (Shimadzu SPD-M20A, Kyoto, Japan) was used as a detector at 450 nm. The *trans*-lycopene and  $\beta$ -carotene were used as standard.

### 2.6 Statistical analysis

This extraction experiment was performed by using a full factorial design and was conducted in triplicate. All data measurements were done in triplicate and expressed as the mean with standard deviation. Duncan's New Multiple Range Test was used for treatment comparison with a significance level of  $p < 0.05$ .

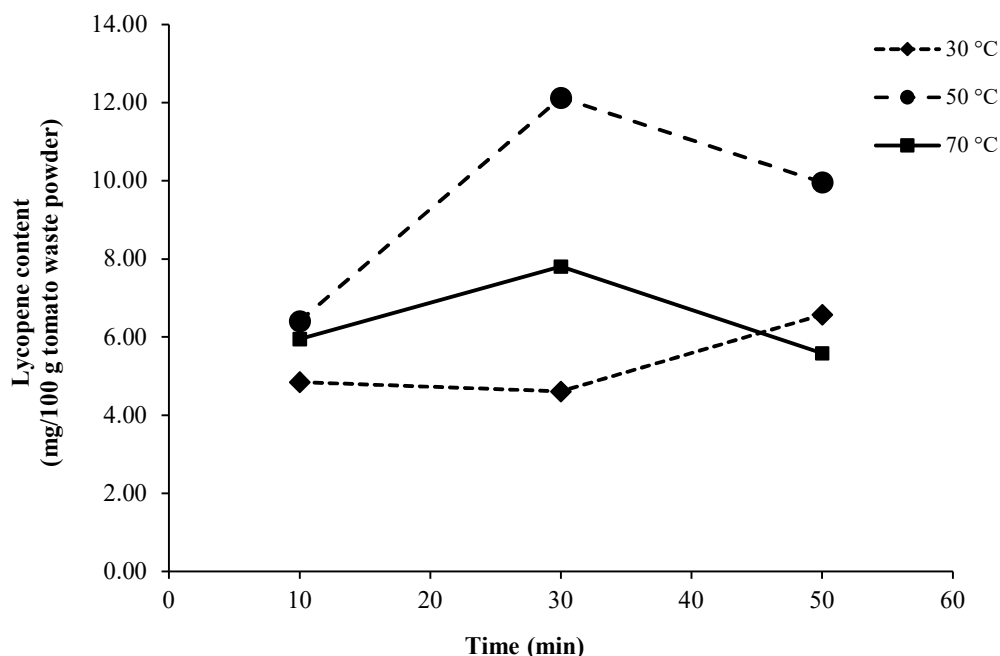
## 2.7 Fuzzy assessment analysis

A fuzzy assessment analysis was performed in order to determine the most suitable conditions for lycopene extraction using the ultrasound-assisted method. The FAM was used to calculate the overall performance index from 4 criteria: 2 quantity criteria were *trans*-lycopene and  $\beta$ -carotene yields, and 2 quality criteria were used, which were the values for DPPH radical scavenging activity and inhibition lipid peroxidation. The performance index for each extraction condition was calculated according to the FAM [18].

## 3. Results and discussion

### 3.1 Ultrasound-assisted extraction condition and lycopene content

Industrial tomato waste was derived from tomato paste industry and our previous studies [16] reported that the protein, crude fat, crude fiber, ash and carbohydrate content was  $22.81 \pm 0.24$ ,  $11.89 \pm 0.56$ ,  $54.61 \pm 0.78$ ,  $2.75 \pm 0.02$  and  $7.88 \pm 1.12\%$  (%dry basis), respectively. It was used as a material for carotenoid extraction. Extraction was performed under different ultrasound-assisted extraction (UAE) conditions, namely different extraction temperatures (30, 50, and 70 °C) and different times (10, 30, and 50 min). The lycopene yield of each crude extract was calculated by using an absorbance at 503 nm and an extinction coefficient of lycopene in hexane. The lycopene content in each condition is shown in Figure 1.



**Figure 1** The lycopene content of crude extract from tomato waste powder.

The results showed that extraction temperature and time affected lycopene yields. An extraction temperature of 50 °C gave a higher lycopene yield than that of 30 °C. This could be due to the higher extraction temperature enhanced the solubility of lycopene in the solvent medium. Nevertheless, an extraction temperature of 50 °C yielded a higher amount of lycopene than that of 70 °C. This can be explained by the cavitation effects during sonication. The gaseous micro-bubbles were generated during sonication and it continually collapsed onto the surface of the material and micro-jet could occur on the surface, which was referred to a cavitation effect [3]. The cavitation effects together with a high temperature would enhance the release of the extract into the solvent. However, the higher temperature causing a higher vapor pressure of the solvent, which in turn would influence the cavitation phenomenon and thus a decrease in efficiency of the extraction [3]. Therefore, the extraction temperature of 70 °C which was closed to the boiling point of solvent was the condition in which the cavitation effect may less effect compare to other temperature regarding to the lower lycopene. This trend was also found in ultrasound-assisted extraction of lycopene from red grapefruit [3]. In the present study, the lycopene yield of crude extract under the extraction temperature of 70 °C was comparable to that of 30 °C. Furthermore, the longer extraction time, the lower extracted lycopene, especially at extraction temperature of 70 °C, this probably

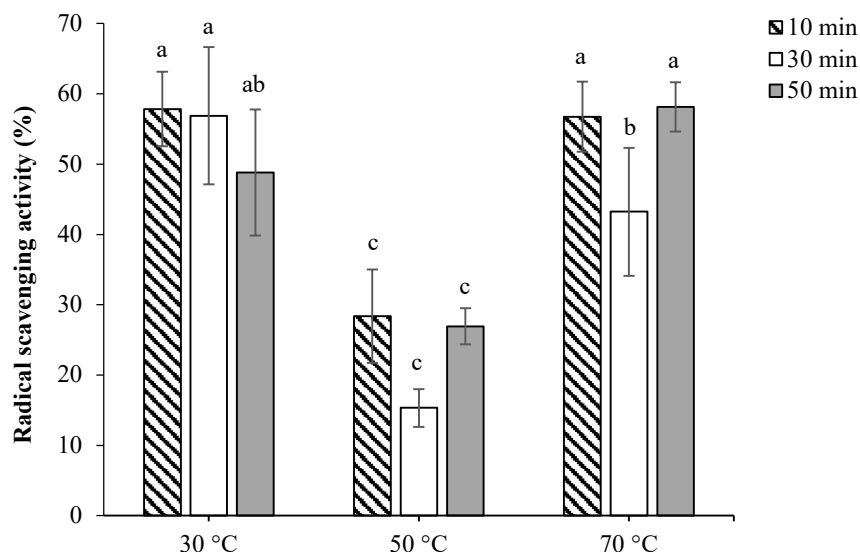
is as a result of the degradation of lycopene. However, the extraction time of 50 and 30 min was not significantly difference. It can be assumed that all lycopene was almost released from the material at the extraction time of 30 min.

In this study, the extraction condition yielding the highest lycopene content was the extraction temperature of 50 °C for 30 min. This condition yielded 12.13 mg lycopene/100 g of tomato waste powder. This yield was higher than that produced by tomato processing waste skin in the research conducted by Kaur et al. [13], for which extraction was the highest at 50 °C for 8 min (1.98 mg/100 g), as well as that produced by tomato pulp waste in research conducted by Poojary and Passmonti [3], for which extraction was the highest at 20 °C for 40 min (3.6 mg/100 g). However, the lycopene yield in the present study was lower than that produced by extraction from tomato skin using a microwave at 400 W for 60 s, which yielded 13.592 mg of all-*trans*-lycopene per 100 g [27], as well as that produced by extraction from tomato pulp with skin using ultrasonication at 47.6 °C for 45.6 min, which yielded 5.11 mg of all-*trans*-lycopene per g [2]. This is probably due to a number of factors differentiating the studies, namely the extraction method, extraction conditions (temperature, time, solid/solvent ratio), and the type of extraction solvent used, as well as the composition of the raw material. In the present study, the tomato waste used as a raw material was derived from the tomato paste industry, extraction was performed in a sonication bath, and the extraction solvent was 95% ethanol and fractionation solvent were *n*-hexane.

### 3.2 Antioxidant activity of crude extract

#### 3.2.1 DPPH radical scavenging activity

Carotenoids are reported to have a high potential for free radical scavenging. Moreover, it has been reported that the DPPH radical scavenging activity method was suitable for investigating oils [28]. Therefore, DPPH radical scavenging activity of the crude extracts were measured.



**Figure 2** Radical scavenging activity (%) of crude extract from tomato waste powder. (Different letter in each bar shows the significant difference ( $p < 0.05$ ) of the values).

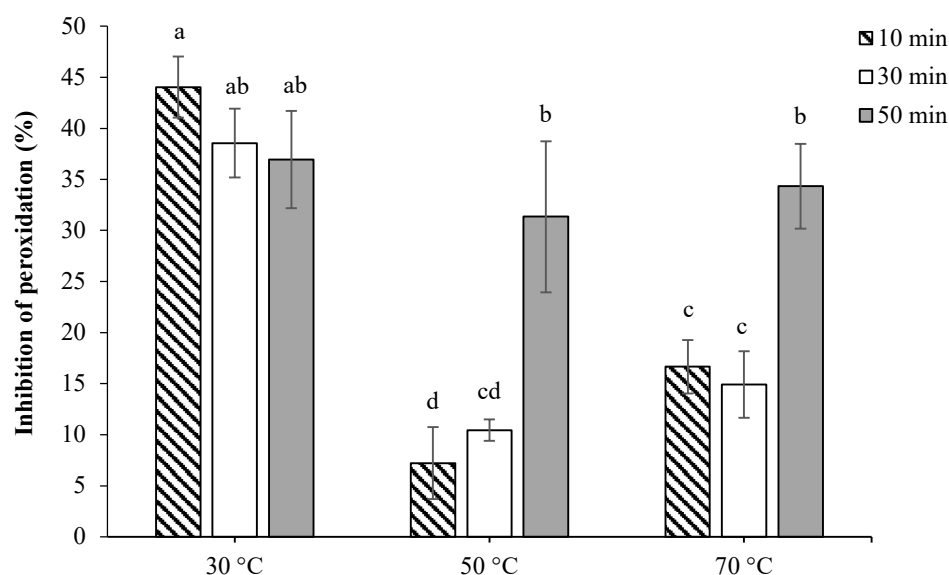
The radical scavenging activity values for the different crude extracts are shown in Figure 2. The results show that the extraction temperature more affect to its activity than extraction time. The radical scavenging activity value of the extract can be divided into three categories: low, medium, and high level. The extraction condition that yielded low activity level was the extraction temperature of 50 °C for all extraction times. Moreover, the radical scavenging activity of the samples extracted at 70 °C as well as at 30 °C showed higher levels of activity than that at 50 °C. This trend was also found in the research conducted by Yi et al. [5], which found that the antioxidant activity of lycopene-rich extract from tomato skin using higher temperature extraction was lower than that using lower temperature. The extraction conditions that yielded significantly high activity level were the extraction temperature of 30 °C for 10 and 30 min and that of 70 °C for 10 and 50 min.

The antioxidant activity of the lycopene extracts could have been affected by many factors, such as the amount of lycopene, the structure of isomers of the lycopene, and other carotenoid content [5,14]. As a result of lycopene's long chain of conjugated double bonds, oxidation can be easily increased due to peroxide radicals in

the conjugate position [29,30]. Moreover, it is easily isomerized during cooking and extraction [30]. Hackett et al. [31] reported that lycopene in tomato oleoresin is oxidized in the temperature range of 25-50 °C, while it is isomerized rapidly in the temperature range of 75-100 °C. In this study, the extraction temperature was kept lower than the isomerization temperature range. However, the temperature range of 25-50 °C the lycopene was predominately oxidized which was not affected to lycopene composition [5]. In the other hand, Oliveira et al [32] reported that the lycopene in alcohol solution was stable in thermo-sonication at temperature up to 60 °C, however, lycopene in tomato puree was reduced which was due to high hydrogen peroxide concentration during plant stress. This hydrogen peroxide level was generated to peroxy radical which may be affected to its activity. Moreover, the cavitation phenomenon can generate reactive hydroxyl radicals, even in the presence of a small amount of water [3] hence generated hydroxyl radicals could be affected to the DPPH radical scavenging activity for the extract yielded at the temperature of 50 °C, at which a stronger cavitation effect was observed. Moreover, the cavitation effect may be influenced the isomerization of *trans*-lycopene into different *cis*-lycopene and *cis*-lycopene show lower activity [3]. Besides, the antioxidant activity of crude extract may be affected by other compounds which can be released during Ultrasound-Assisted Extraction. Kahili et al. [11] stated that lycopene was the main component for radical scavenging, however,  $\beta$ -carotene had a significant effect with lycopene in order to antioxidant properties, therefore, the characteristics of crude extract were determined using HPLC and will be discussed later. On the other hand, even though the UAE produced hydroxyl radicals, the extract still showed DPPH radical scavenging activity. The effects of this phenomenon can be minimized by using an oxygen-free nitrogen atmosphere, according to Eh and Toeh [2].

### 3.2.2 Inhibition of lipid peroxidation

Besides DPPH radical scavenging activity, inhibition of lipid peroxidation of each crude extract was also measured by using a ferric thiocyanate test, which was used to determine the peroxide-forming inhibition capacity of the compounds in the oil system [26]. Inhibition of lipid peroxidation was expressed as percentages comparing each extract to the control mixture. The results are shown in Figure 3.



**Figure 3** Inhibition of lipid peroxidation (%) of crude extract from tomato waste powder. (Different letter in each bar shows the significant difference ( $p < 0.05$ ) of the values).

Figure 3 shows that the extraction temperature of 30°C and extraction time of 10 min yielded the highest value for inhibition of lipid peroxidation by a significant amount (44.03%), while the extraction temperature of 50 °C and extraction time of 10 min yielded the lowest (7.21%). This finding is consistent with findings for DPPH radical scavenging activity and may be explained by the effect of the cavitation phenomenon. Meanwhile, the extraction temperature of 70 °C resulted in medium levels of inhibition of lipid peroxidation, lower than those measured of the extracts produced at 30 °C. Hence, the differences are probably due to the thermal degradation or isomerization effect during extraction.

However, similar to DPPH radical scavenging activity, values for inhibition of lipid peroxidation were not in accordance with the amount of lycopene in the crude extract. This shows that the stability or structure of

lycopene may play a more important role than the amount of lycopene. Moreover, other antioxidant compounds which may be extracted during sonication can be affected to its antioxidant activity.

### 3.3 High performance liquid chromatography analysis

To identify the lycopene and  $\beta$ -carotene content in the crude extract, a HPLC was performed and the retention time was compared to *trans*-lycopene and  $\beta$ -carotene standards. The quantities of *trans*-lycopene and  $\beta$ -carotene in the crude extracts are shown in Table 1.

The results show that each crude extract contained both *trans*-lycopene and  $\beta$ -carotene in the ratio range of 0.7-1.2 (*trans*-lycopene/ $\beta$ -carotene). This was due to the extraction solvent used. The solvent used for lycopene extraction was commonly an organic solvent, such as ethanol, acetone, ethyl acetate, hexane, petroleum ether, or chloroform, and its mixture [13]. Calvo et al. [33] reported that the use of ethanol as a lycopene extraction solvent produced a greater yield compare to ethyl acetate. Poojary and Passamonti [3] used 25% acetone in *n*-hexane for lycopene extraction, which yielded 98.3% purity for all-*trans*-lycopene. Eh and Teoh [2] used the mixture of *n*-hexane/acetone/ethanol for lycopene extraction from tomatoes with an ultrasonication technique, yielding 98.27% purity for all-*trans*-lycopene.

Similar to the findings related to lycopene yields detected by UV visible spectrophotometer, the amount of lycopene detected by HPLC also showed that the extraction temperature of 50 °C yielded higher amounts of lycopene, as well as  $\beta$ -carotene, compared to that of 30 °C. This can be explained in a similar way as previously described, by the high extraction temperature coupled with the high cavitation effect at the temperature of 50 °C. The temperature of 70 °C yielded a lower amount of *trans*-lycopene and  $\beta$ -carotene compared to that of 50 °C because the higher vapor pressure at the higher temperature (70 °C) reduced the cavitation effect. Moreover, the amounts of  $\beta$ -carotene in the crude extract produced at temperatures of 30 and 70 °C could not be differentiated clearly. This could be explained by cavitation phenomenon during sonication, which can improve the yield of carotenoid extraction in the middle range of temperature in this study.

**Table 1** *Trans*-lycopene and  $\beta$ -carotene contents and ratio in crude extract.

Extraction temperature (°C)	Extraction Time (min)	Trans-lycopene (mg/100 g tomato waste powder)	$\beta$ -carotene (mg/100 g tomato waste powder)
30	10	1.91 $\pm$ 0.52 <sup>d</sup>	2.86 $\pm$ 1.10 <sup>e</sup>
	30	2.23 $\pm$ 0.21 <sup>d</sup>	2.58 $\pm$ 0.19 <sup>e</sup>
	50	2.64 $\pm$ 0.29 <sup>d</sup>	3.73 $\pm$ 0.10 <sup>de</sup>
50	10	5.41 $\pm$ 0.59 <sup>b</sup>	6.00 $\pm$ 0.72 <sup>b</sup>
	30	9.23 $\pm$ 0.26 <sup>a</sup>	7.80 $\pm$ 0.01 <sup>a</sup>
	50	5.98 $\pm$ 0.79 <sup>b</sup>	5.56 $\pm$ 1.04 <sup>bc</sup>
70	10	2.78 $\pm$ 0.64 <sup>d</sup>	3.24 $\pm$ 0.61 <sup>e</sup>
	30	4.27 $\pm$ 0.87 <sup>c</sup>	4.68 $\pm$ 0.61 <sup>cd</sup>
	50	3.07 $\pm$ 0.51 <sup>d</sup>	2.71 $\pm$ 0.15 <sup>e</sup>

\*Different letter in each column shows the significant difference ( $p < 0.05$ ) of the values.

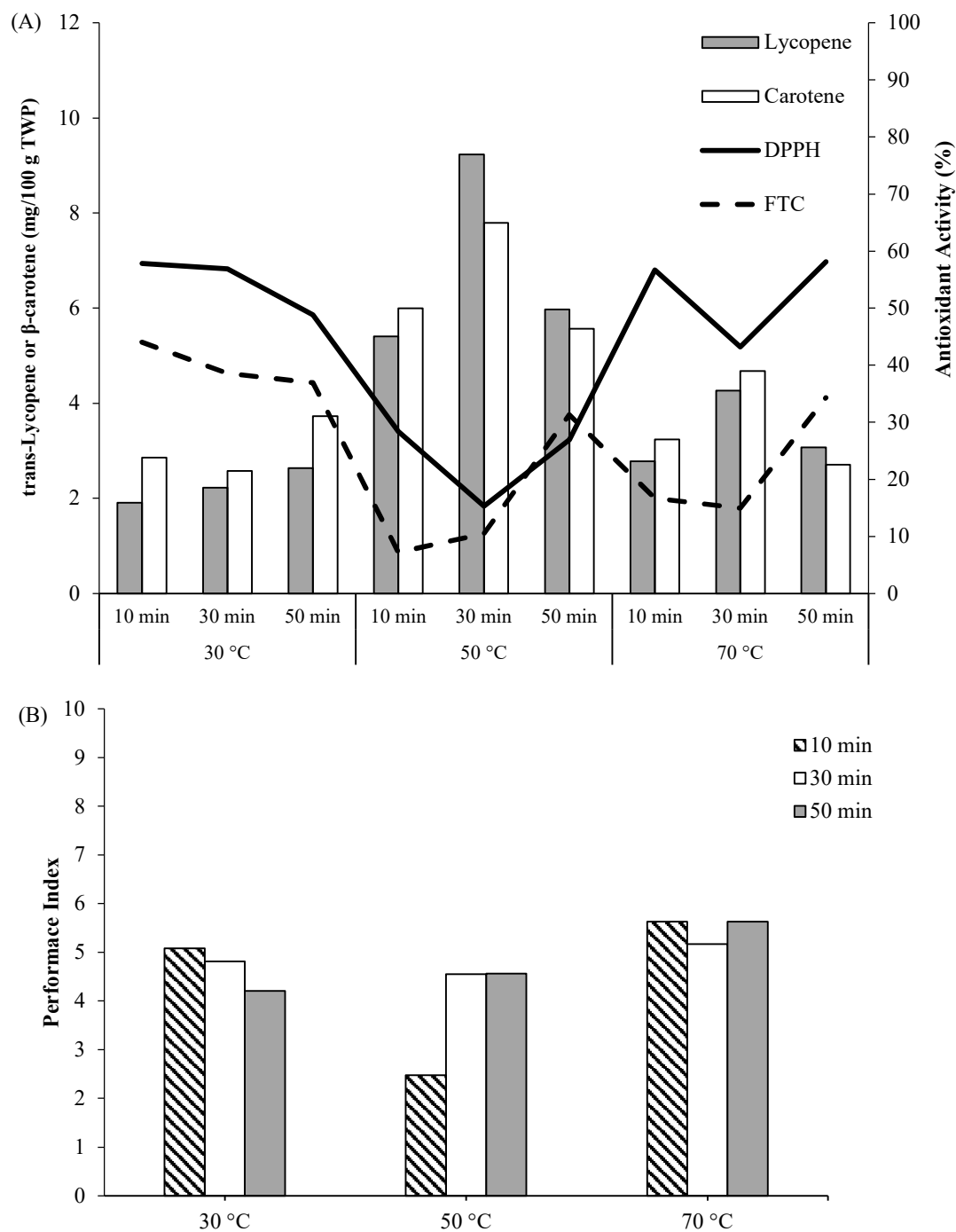
### 3.4 Fuzzy assessment analysis

The various thermo-sonication conditions that yielded high quantities did not show strong correlations to its antioxidant activity which can be seen in (Figure 4 (A)). This result can be pointed that the more cavitation effects, the more hydroxyl radical generation, and the lesser antioxidant activity of crude extracts. This result was also found by extraction using supercritical CO<sub>2</sub> which the highest yield was found at the extraction temperature of 80 °C while the lowest DPPH activity was found at the extraction of 40 °C [34]. Moreover, Oliveira et al. [32] the thermal-sonication at 60 °C of tomato puree decreased the lycopene content. Therefore, in order to identify the suitable extraction condition, yielding both high lycopene content and high levels of radical scavenging activity and inhibition of lipid peroxidation, the fuzzy set theory was applied as a mathematical decision method.

The FAM can evaluate two or more important criteria and offers a numerical value to judge the best solution. Therefore, in this study, the FAM was applied in order to choose the best extraction conditions while considering 4 criteria for the crude extract. The *trans*-lycopene and  $\beta$ -carotene content which was determined by using an HPLC were used as a quality criterion. On the other hand, antioxidant activity was measured by using 2 methods, DPPH radical scavenging activity and inhibition of lipid peroxidation were used as a quantity criterion. The resulting measurements were thus applied as quality criteria for the crude extract. In order to obtain a performance score in the range of (0,10), the minimum and maximum values for each criterion were used as lower and upper bounds, respectively. The weighting of the 4 criteria, 2 of which evaluated quantity of the extract and 2 of which evaluated quality, is also an important aspect in calculating the performance index of each condition. According to the lycopene was the main component for radical scavenging [11], therefore the

weighting of quality criteria was set at 30:20 for *trans*-lycopene and  $\beta$ -carotene yield, respectively. Meanwhile, the weighting of both antioxidant activities was set at 25:25. The performance score and performance index were calculated by using a Scilab Software Package developed by Lasunon [18]. The performance index for each test in the experiment was calculated and is presented in (Figure 4 (B)).

In our study, both quantity and quality of the extracts were considered before choosing the most suitable extraction conditions. According to the highest performance index, the best extraction condition was the extraction temperature of 70 °C for 10 min.



**Figure 4** (A) The carotenoid content and antioxidant activity and (B) performance index of each extraction condition.



#### 4. Conclusion

Many works have been attempted to find the novel extraction method and condition that gave the highest yield; however, the quality of extracts was also important for further used. Therefore, the FAM technique was applied to find the optimum ultrasound-assisted condition in order to extract lycopene from industrial tomato waste with optimum antioxidant activity. The lycopene yield was affected by extraction temperature and time in which the extraction temperature of 50 °C and extraction time of 30 min produced the highest yield of trans-lycopene and  $\beta$ -carotene which probably was due to stronger cavitation effect than the other, as well as a higher temperature resulting in enhancing the solubility. The antioxidant activity of each crude extract was measured by using DPPH radical scavenging activity and inhibition of lipid peroxidation. The extraction temperature of 70 °C for 50 min yielded the highest levels of DPPH radical scavenging activity, while yielded the highest values for inhibition of lipid peroxidation. The carotenoid content was not strong correlation with their amounts. The 4 evaluation criteria, trans-lycopene and  $\beta$ -carotene yield, DPPH radical scavenging activity level, and inhibition of lipid peroxidation level, were assigned the weights of 30, 20, 25, and 25, respectively. The highest performance index, calculated by using triangular fuzzy theory, was found at the extraction temperature of 70 °C for 10 min. This extraction condition gave the highest yield of active compounds with high quality antioxidant activity.

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